InvivoGen insight

SPRING 2017

SUMMARY :

REVIEW

Mitochondrial DNA in innate immunity and autophagy

PRODUCTS

Autophagy Reporter Cells

- HeLa-DiFluo™ cells
- RAW-DiFluo™ cells
- THP1-DiFluo™ cells

Autophagy Inducers & Inhibitors

Antimicrobial Agent for Primary Cells

- Primocin™

InvivoGen

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Mitochondrial DNA in innate immunity and autophagy

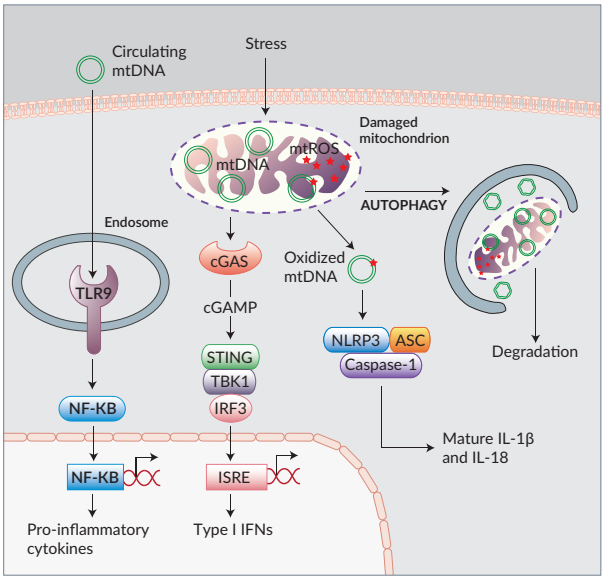
Mitochondria are unique mammalian organelles known as the energy factories of the cell and believed to have evolved from aerobic bacteria. They are important for cellular metabolism and apoptosis. Growing evidence suggests that they also play a central role in innate immunity and may contribute to inflammatory diseases. Following infection and stress, damaged mitochondria release their constituents, including mitochondrial DNA (mtDNA), which acts as a potent danger-associated molecular pattern (DAMP). It induces inflammatory responses mediated by various pattern recognition receptors (PRRs). The major PRRs involved in mtDNA recognition are Toll-like receptor 9 (TLR9), Nod-like receptor family, pyrin domain containing 3 (NLRP3) and cyclic GMP-AMP (cGAMP) synthase (cGAS).

Mitochondrial DNA resembles bacterial DNA, as it contains unmethylated CpG dinucleotide repeats, which are recognized by the endosomal receptor TLR9. Several studies have demonstrated that mtDNA released in the circulation after injury directly activates TLR9, causing the activation of NF-κB and the production of pro-inflammatory cytokines such as TNF-α and IL-6[1-3]. Blockade of TLR9, either by using TLR9 inhibitory ODNs[1, 2] or by deleting the *Tlr9* gene[4], has been shown to attenuate the inflammatory response, thus confirming the involvement of TLR9 in the recognition of mtDNA.

Another inflammatory pathway activated by mtDNA is the one triggered by formation of the NLRP3 inflammasome that results in caspase-1-dependent secretion of the inflammatory cytokines IL-1β and IL-18. Recent data suggest that mtDNA released in the cytosol induces the activation of the NLRP3 inflammasome. Depletion of mtDNA resulted in the inhibition of IL-1β and IL-18 secretion in cells stimulated with inflammasome inducers[5]. Furthermore, oxidized mtDNA was found to directly bind and activate NLRP3[6].

Mitochondrial DNA is also inflammatory by engaging the cGAS-STING pathway to initiate the production of type I interferons (IFNs). Recently, mtDNA released in the cytosol of cells undergoing apoptosis was shown to activate cGAS to recruit stimulator of interferon genes (STING) through the second messenger cGAMP[7,8]. STING induces type I IFN transcription via the TBK1–IRF3 signaling axis. This pathway was also recently found to be activated in response to mtDNA stress induced by herpesvirus infection[9].

The inflammatory response triggered by mtDNA is closely linked to autophagy. Autophagy is a potent adaptive mechanism that protects cells from stress-induced injury by removing unwanted cytosolic contents (e.g. DNA, proteins, mitochondria and intracellular pathogens) through their sequestration in a double-membraned vacuole, the autophagosome, and their subsequent degradation following fusion with lysosomes. In addition, autophagy can limit excessive inflammatory response by modulating the activity of key immune mediators, such as NF-κB[10]and STING[11]. In pathological conditions such as microbial infection or major trauma, dysfunctional mitochondria and damaged mtDNA accumulate in the cytosol to a level exceeding the degradative capacity of autophagy, thus resulting in the loss of its negative regulatory function. This results in increased inflammatory responses, often accompanied by chronic inflammation, due to aberrant regulation of TLR9[2,12], NLRP3[5,10] and/or cGAS-STING signaling[13]. Further studies are required to better understand the role of mtDNA in mitochondria-related diseases, including infectious and autoimmune diseases as well as cancer.



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Autophagy Reporter Cells

Autophagy is an essential homeostatic process by which cytoplasmic materials are degraded in lysosomes. This multi-step process involves isolation of cargo within membranes, autophagosome formation, fusion with lysosomes, degradation and recycling of cargo contents. One key protein used to study this 'autophagic flux' is LC3 (microtubule-associated protein 1 light chain 3). This protein is processed and recruited from the cytosol to the isolation membrane. This localization serves as a marker for autophagic membranes and for monitoring the process as it develops. Chimeric proteins consisting of LC3B fused to a green fluorescent protein (GFP) and a red fluorescent protein (RFP) provide a simple means of monitoring the autophagic process. Autophagosomes marked by an RFP-GFP-LC3 show both RFP and GFP signals. After fusion with lysosomes, GFP signals are significantly reduced due to acidic conditions, while RFP signals remain relatively stable.

HeLa-DiFluo™ hLC3 Cells
RAW-DiFluo™ mLC3 Cells
THP1-DiFluo™ hLC3 Cells

HeLa-Difluo™ hLC3 cells, RAW-Difluo™ mLC3 cells and THP1-Difluo™ hLC3 cells are autophagy reporter cells derived from the human HeLa epithelial carcinoma cell line, the murine RAW 264.7 macrophage cell line and the human THP-1 monocytic cell line, respectively. They express an RFP::GFP::LC3 fusion protein, RFP-GFP-LC3, in which the N-terminus of human or mouse LC3 is fused to two fluorescent reporter proteins: an RFP (acid-stable) and a GFP (acid-sensitive). In these cells, the RFP-GFP pair enables monitoring of autophagic flux in real time by detecting the appearance of green GFP-LC3 and/or red RFP-LC3 puncta by fluorescence microscopy. Early in autophagy, both RFP and GFP signals are detected. As the fusion of the autophagosomes with the lysosomes progresses, the GFP fluorescence diminishes, leaving only the RFP fluorescence visible. The percentages of GFP-LC3/RFP-LC3 positive and of RFP-LC3 positive cells can be determined and these values can be used to assess autophagic flux, using methods described previously[1,2].

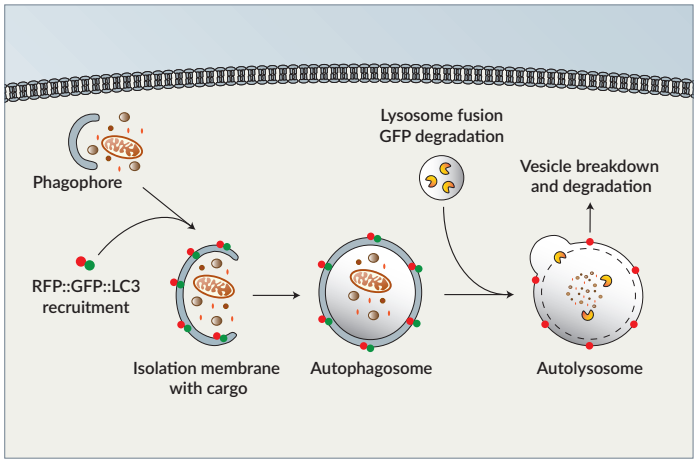
HeLa-Difluo™ hLC3 cells, RAW-Difluo™ mLC3 cells and THP1-Difluo™ hLC3 cells are resistant to Zeocin™.

1. Loos B. *et al.* 2014. Defining and measuring autophagosome flux- concept and reality. Autophagy. 2014;10(11):2087-96. 2. Kimura S. *et al.*, 2007. Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent tagged LC3. Autophagy. 3(5):452-60.

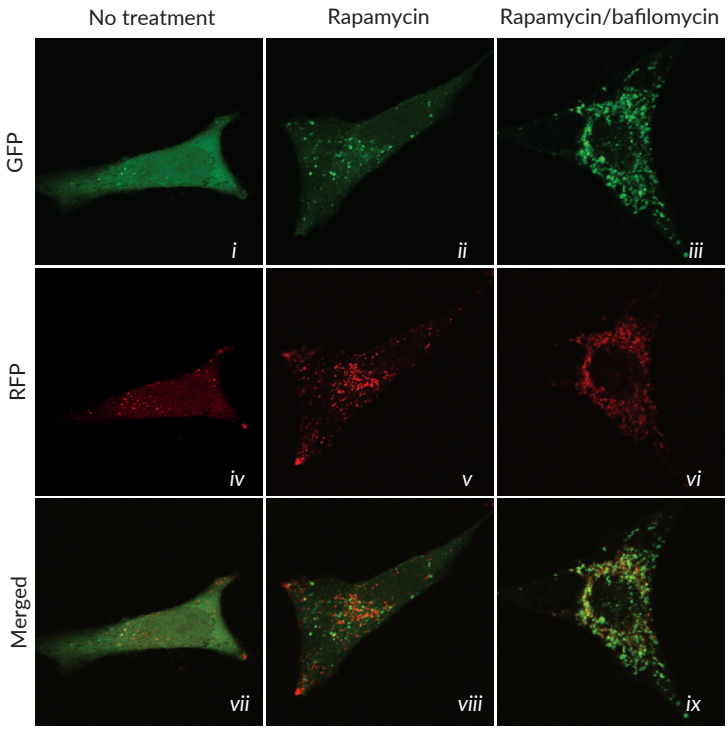
PRODUCT	QUANTITY	CAT. CODE
Hela-DiFluo™ hLC3 cells	3-7 x 10^6 cells	heldf-hlc3
RAW-DiFluo™ mLC3 cells	3-7 x 10^6 cells	rawdf-mlc3
THP1-DiFluo™ hLC3 cells	COMING SOON	

RELATED PRODUCTS

PRODUCTS	DESCRIPTION	CAT. CODE
Zeocin™	Selective antibiotic	ant-zn
pSELECT-GFP-hLC3	Expression plasmid	psetz-gfplc3
pSELECT-GFP-mLC3	Expression plasmid	psetz-gfpmlc3



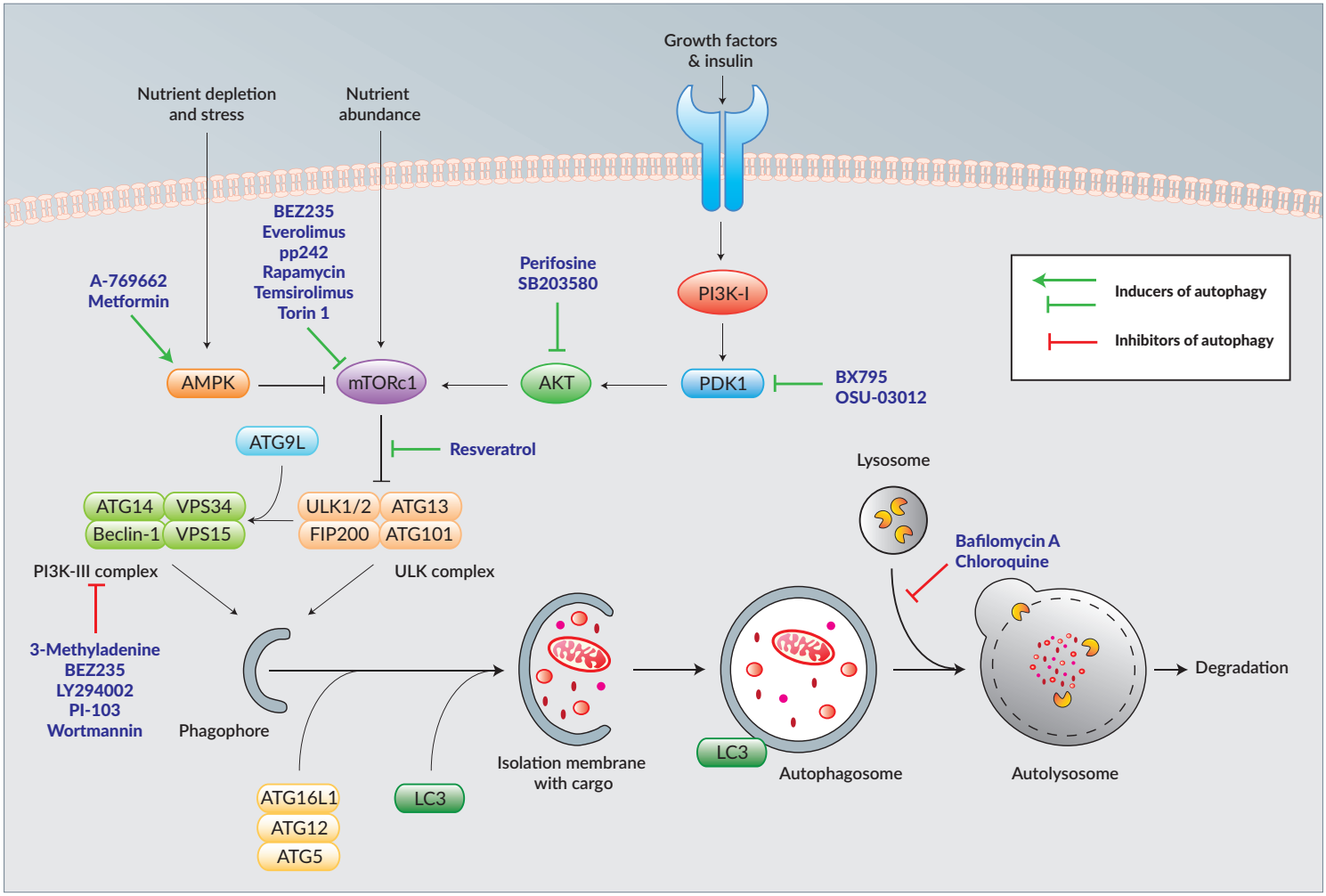
Detection of fluorescent puncta in HeLa-DiFluo hLC3 cells



HeLa-DiFluo™ cells were treated with 25 µM rapamycin alone or with 25 µM rapamycin and 500 nM bafilomycin A1 (to inhibit autophagosome/lysosome fusion). After 24 hour incubation, the cells were fixed with 2% PFA and analyzed by confocal microscopy. Note that both yellow (autophagosome) and red (autolysosome) puncta increase in panel *viii*, whereas most puncta in panel *ix* are yellow (autophagosome).

Autophagy Inducers & Inhibitors

Autophagy is the primary catabolic program activated by cellular stressors including nutrient and energy starvation. It begins by the production of the autophagosome, a step controlled by a suite of proteins including the class III phosphatidylinositol (PI) 3-kinases of the VPS34 complex, followed by its acidification after fusion with the lysosome. Thus autophagy can be blocked by using inhibitors of class III PI3-kinases that suppress autophagic sequestration, or inhibitors of lysosomal acidification that prevent autolysosome formation and autophagic degradation. Autophagy is a highly regulated process. mTORC1, a kinase sensitive to nutrients and growth factors, is a major repressor of autophagy. Direct inhibitors of mTORC1 and those of pathways activating mTORC1 subsequently induce autophagy.



PRODUCT	QUANTITY	CAT. CODE
3-Methyladenine	50 mg	tlrl-3ma
A-769662	10 mg	inh-a769
Bafilomycin A1	10 mg	tlrl-baf1
BEZ235	50 mg	inh-bez2
BX795	5 mg	tlrl-bx7
Chloroquine	250 mg	tlrl-chq
Everolimus (RAD001)	5 mg	tlrl-eve
LY294002	5 mg	tlrl-ly29
Metformin	1 mg	tlrl-metf
OSU-03012 (AR-12)	10 mg	inh-os03

PRODUCT	QUANTITY	CAT. CODE
Perifosine	5 mg	tlrl-peri
PI-103	5 mg	inh-pi10
pp242	5 mg	inh-pp242
Rapamycin (Sirolimus)	5 mg	tlrl-rap
Resveratrol	100 mg	tlrl-resv
SB203580	5 mg	tlrl-sb20
Temsirolimus	10 mg	inh-tems
Torin 1	10 mg	inh-tor1
Wortmannin	5 mg	tlrl-wtm

Antimicrobial Agent for Primary Cells

Primocin™

Primary cell cultures face the constant threat of contamination by destructive microbes, whether from the original cell source (e.g. skin or intestinal samples) or the surrounding environment (e.g. laboratory, equipment, personnel, etc.). To help you protect your primary cells, InvivoGen has developed Primocin™, a proprietary broad-spectrum antibiotic.

Description

Primocin™ eliminates mycoplasma, bacteria and fungi by blocking both DNA and protein synthesis. It acts on targets found only in microorganisms, including three bacterial targets (DNA gyrase and the prokaryotic ribosomal subunits 30S and 50S) and one fungal target (ergosterol, a molecule found exclusively in the cell membrane of fungi and yeasts). Owing to its specific cocktail of antibiotics, Primocin™ offers greater protection than even InvivoGen's own Normocin™ against certain bacteria ubiquitous in laboratory environments, including *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Bacillus* species. Crucially, Primocin™ is non-toxic to primary cells, thanks in part to an antimycotic agent that is less toxic and more stable than Amphotericin B.

Applications and Efficacy

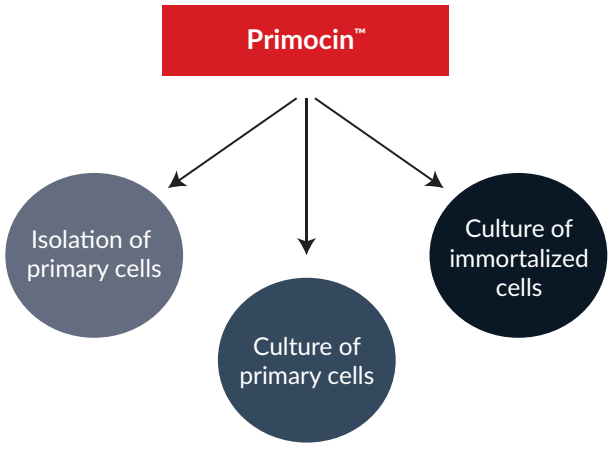
Primocin™ is frequently cited in the literature on primary cell isolation and culture. For example, it has been used during sub-cloning of primary human natural killer (NK) cells isolated from peripheral blood mononuclear cells (PBMCs)[1], and for isolation of astrocytes and neurons from murine embryonic brain tissue[2]. Moreover, recent studies have demonstrated the efficacy of Primocin™ for treatment of stem cells, including in neural induction of human embryonic stem cells corresponding to Fragile X syndrome[3]. In fact, Wang et al., in their development of long-term cultures of human pluripotent stem cells (hPSCs), treated the cells with medium that contained both Primocin™ and InvivoGen's anti-mycoplasmal agent Plasmocin™ to further protect against bacterial and mycoplasmal infection, defining this as a "critical step" during sorting and culture[4]. Several research groups have applied Primocin™ in an emerging domain of applied biology: 3D cell culture and organoids. For example, in a study on endoplasmic reticulum (ER) stress, inflammation and apoptosis in murine colonic organoids, Graham et al. added Primocin™ to all the organoid media[5]. Similarly, Weeber and colleagues used Primocin™ to ensure proper growth of tumor organoids derived from metastasis biopsies collected from colorectal cancer (CRC) patients[6]. Lastly, Nichols et al. employed Primocin™ at two steps during preparation of lung organoids built from primary adult pulmonary cells and pediatric lung scaffolds[7]: treatment of the source lung tissue and subsequent culture of the harvested lung and tracheal/bronchial cells.

Although Primocin™ is perfectly compatible with many standard antibiotics, one of its greatest advantages is that it obviates the need for using them. In fact, Primocin™ is an ideal choice for treating primary cell cultures that are threatened by microbes that cannot be killed with other products. For example, Cao & Poss, in their very recent article on zebrafish heart explant cultures, reported that Primocin™ reduced contamination of primary epicardial cells during the first few days of culture, whereas a combination of Pen/Strep and Fungizone™ (Amphotericin B) failed to do so[8].

Lastly, Primocin™ can also protect immortalized cell lines. For instance, Pastrana et al. treated 293TT, ART, SFT and A549 cell lines with the antimicrobial agent before screening them for BK polyomavirus infectivity[9].

Researchers around the world trust Primocin™ to protect their primary cells from invasive mycoplasma, bacteria and fungi. Be sure to check out InvivoGen's other broad-spectrum antimicrobial agents (see Related Products).

Applications of Primocin™



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PRODUCT	QUANTITY	CAT. CODE
Primocin™	500 mg (10x1 ml)	ant-pm-1

RELATED PRODUCTS

PRODUCTS	DESCRIPTION	CAT. CODE
Normocin™	Bacteria prevention	ant-nr
Normocure™	Bacteria elimination	ant-noc
Plasmocin™ prophylactic	Mycoplasma prevention	ant-mpp
Plasmocin™ treatment	Mycoplasma elimination	ant-mpt